Supplemental Material

This Supplemental Material includes 1) individual GABA spectra, 2) estimation of GABA-macromolecule contamination and glutamate-glutamine separation, and 3) relationships between entire DMN deactivation and neurotransmitters.

1. Individual GABA spectra

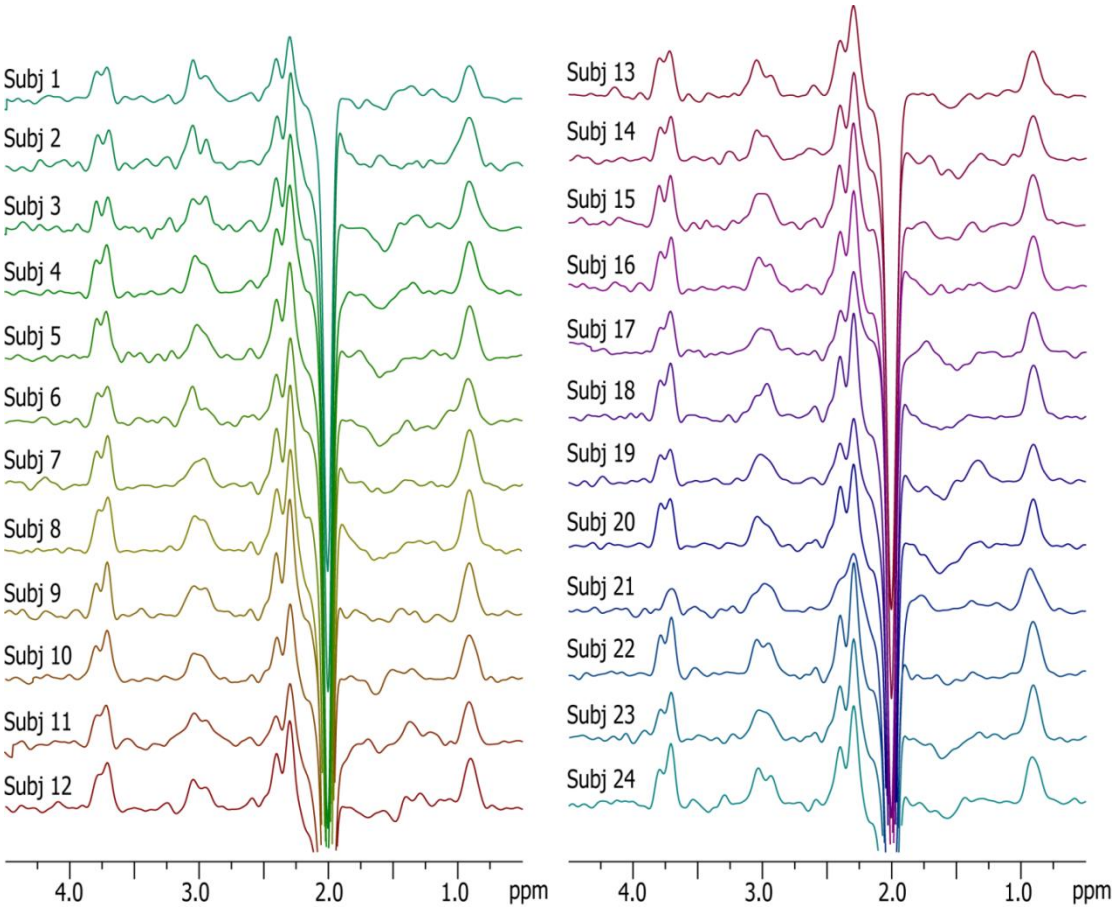

Figure S1. All spectra were zero-filled to 8192 points and a weighting function of Lorentzian–Gaussian transformation (lb = -3 Hz, gb = 8 Hz) was applied. After Fourier transform, it was phased by zero-order for -177 ± 4 degrees and first-order for -4.2 ± 2.0 degrees/ppm (the phasing parameters of edit-off spectra were calculated and used as constraints for the difference spectra in LCModel. The displayed peak intensities were normalized to NAA.

2. GABA-macromolecular contamination and glutamate(Glu)/glutamine(Gln) separation

1). **GABA-macromolecular contamination**

The macromolecular (MM) co-editing is an important issue for the GABA detection using editing sequences. The degree of MM signal contamination varies between different sequences and different editing pulses (Henry et al., 2001; Terpstra et al., 2002; Near et al., 2011). In our case, the editing pulses had a length of 19.9 ms for the

bandwidth of 45 Hz.

To estimate the selectivity of the editing pulses, we performed a series of MEGA-PRESS experiments with changing edit frequency from 1.9 ppm to 1.3 ppm with a step of 0.1 ppm. The GABA peak integral at 3.0 ppm as a function of the edit frequency is shown in Figure S2. The curve is similar to the case of 20-ms editing pulses in a previous study (Edden et al., 2012). The co-editing effect at 0.2 ppm offset (the offset of MM) is around 50%. According to GABA and contaminant concentrations in different tissues estimated by Choi et al (2007) and the average tissue compositions in our study, a rough estimate can be made that the contaminant percentage was about 30% in the GABA+contaminant (GABA+) signal. The MM signal at 3.0 ppm estimated from the LCModel fitting of the edit-off spectrum (MM20, CRLB = 16 ± 2%) had no significant correlation with the GABA+ signal ($r = 0.29$, $p = 0.16$). According to previous studies (Hofmann et al., 2001; Mader et al., 2002), the macromolecular concentrations in cortical regions of healthy adults are very stable with respect to age and gender. Therefore, it is likely that the individual differences in GABA+ levels reflected primarily the differences in the GABA concentration (Donahue et al., 2010).

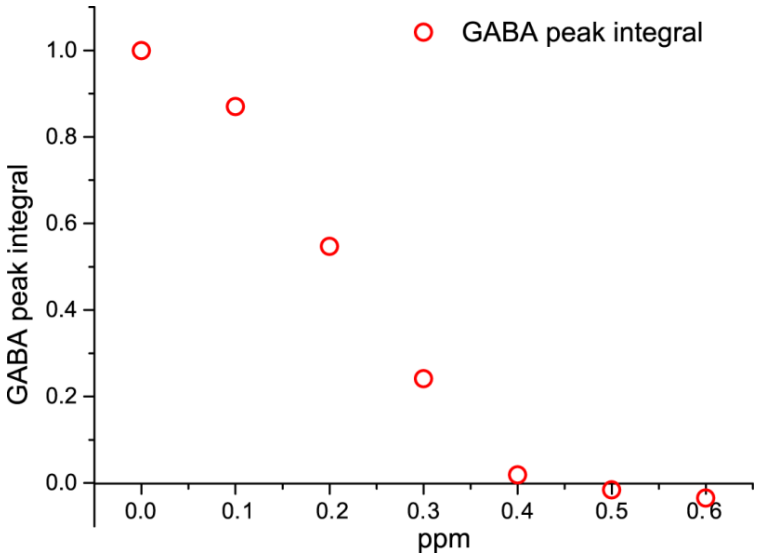

Figure S2. The GABA peak integral obtained from MEGA-PRESS as a function of the editing frequency. The same MEGA-PRESS sequence as in the main study was used on a 10-mM GABA phantom. The edit-off frequency was fixed on 7.5 ppm. The GABA peaks at 3.0 ppm were integrated from 3.3 ppm to 2.7 ppm and normalized to the one with the editing frequency on 1.9 ppm. The x-axis is the frequency offset to 1.9 ppm.

2). Glu and Gln separation

Intracellular Glu in the human brain has different concentrations and functions in glutamatergic neurons, astroglias and GABAergic neurons respectively. Glu has the largest pool in glutamatergic neurons (de Graaf et al., 2011). However, 1H-MRS measurements of Glu, such as used in this study, do not distinguish between metabolic and neurotransmitter pools (Yang et al., 2009). On the other hand, as the

relationship between neuronal glucose oxidation and Glu/Gln cycling is thought to be linear (de Graaf et al., 2004), it is likely that the individual differences in the measured Glu levels also reflect a variation in Glu neurotransmission.

Attached is a representative LCModel fitting spectrum with subtraction of the baseline and with the basis-functions of Glu and Gln. They overlap for about 25% at around 2.4 ppm (Yang et al., 2008) but Glu contributes to most of the signals at the peaks around 2.3 ppm and the Gln signal at around 2.5 ppm has little contamination of Glu. The CRLBs of Glu and Gln were 5 ± 1% and 14 ± 2%, respectively, showing both of them were quantifiable in the LCModel. According to literatures (Mullins et al., 2008; Hancu, 2009), PRESS with TE around 30 ms achieves one of the most repeatable Glu measurements among the state-of-the-art MRS sequences.

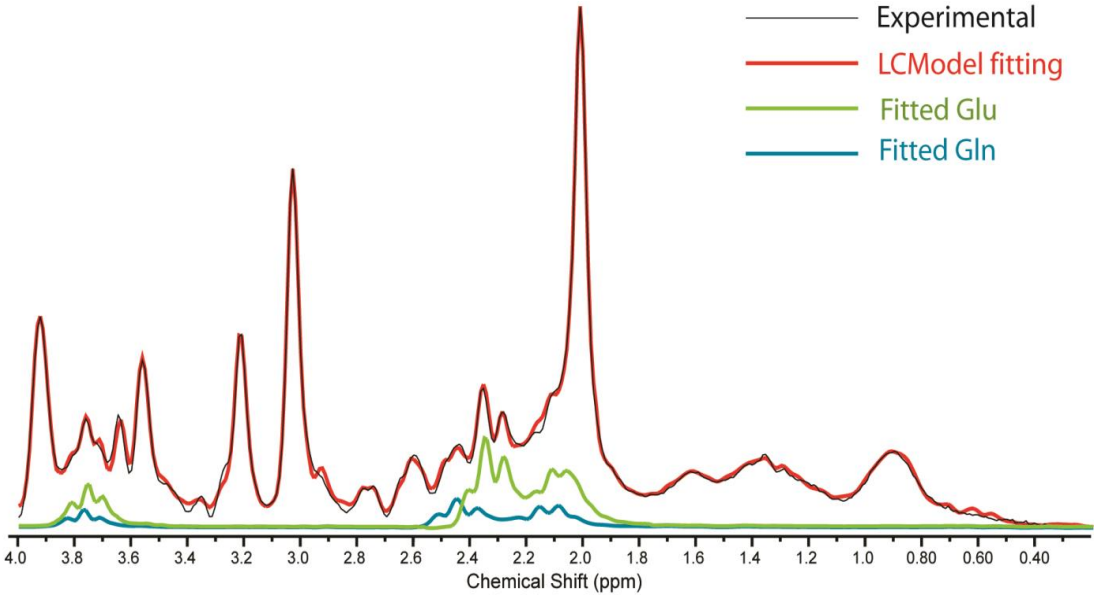

Figure S3. A representative spectrum of a 30-ms PRESS with LCModel fitting and subtraction of baseline, along with Glu and Gln basis sets.

The Glu and Gln combination (Glx) significantly correlated with both Glu(r=.696, p =.0002) and Gln(r=.595, p=.002), but there was no correlation between Glu and Gln (r=-.16, p=.45). Also, there was no significant bivariate correlations between PCC/PCu BOLD signal changes and Glx (p>.45), neither for Gln(p>.14) nor Glu(p>.11). When we used Gln to replace Glu in model 2, its regression coefficients were not significant (1b: t=-1.084, p=.29; 2b: t=-1.18, p=.25; 3b: t=-.26, p=.80). When we used Glx to replace Glu in model 2, its regression coefficients were not significant at 1b and 2b (1b: t=1.56, p=0.13; 2b: t=1.55, p=.14) but significant at 3b (t=2.62, p=.017). These results suggest that it is Glu (not Glx or Gln) that works with GABA in a coordinative way to modulate task-induced BOLD signal change.

3. Complementary analysis on neurotransmitter effects on the entire DMN

deactivation

To test whether PCC/PCu neurotransmitters associate with the entire DMN deactivation, we carried out a complementary analysis. The entire DMN was collectively defined as deactivated regions in the working memory task, mainly including MPFC, PCC/PCu and bilateral parahippocampus. The same regression models used to examine relationship between PCC/PCu deactivation and neurotransmitters were employed to examine relationship between the entire DMN deactivation and neurotransmitters. As shown in Table S1, models including of Glu and GABA significantly explained more variations in the entire DMN BOLD signal change at all cognitive levels. Their coefficients, as shown in Table S2, are relatively lower than that in the regression on PCC/PCu signals. Association patterns, as shown in the partial plots in Figure S4, were consistent with that in PCC/PCu region only (Figure 2).

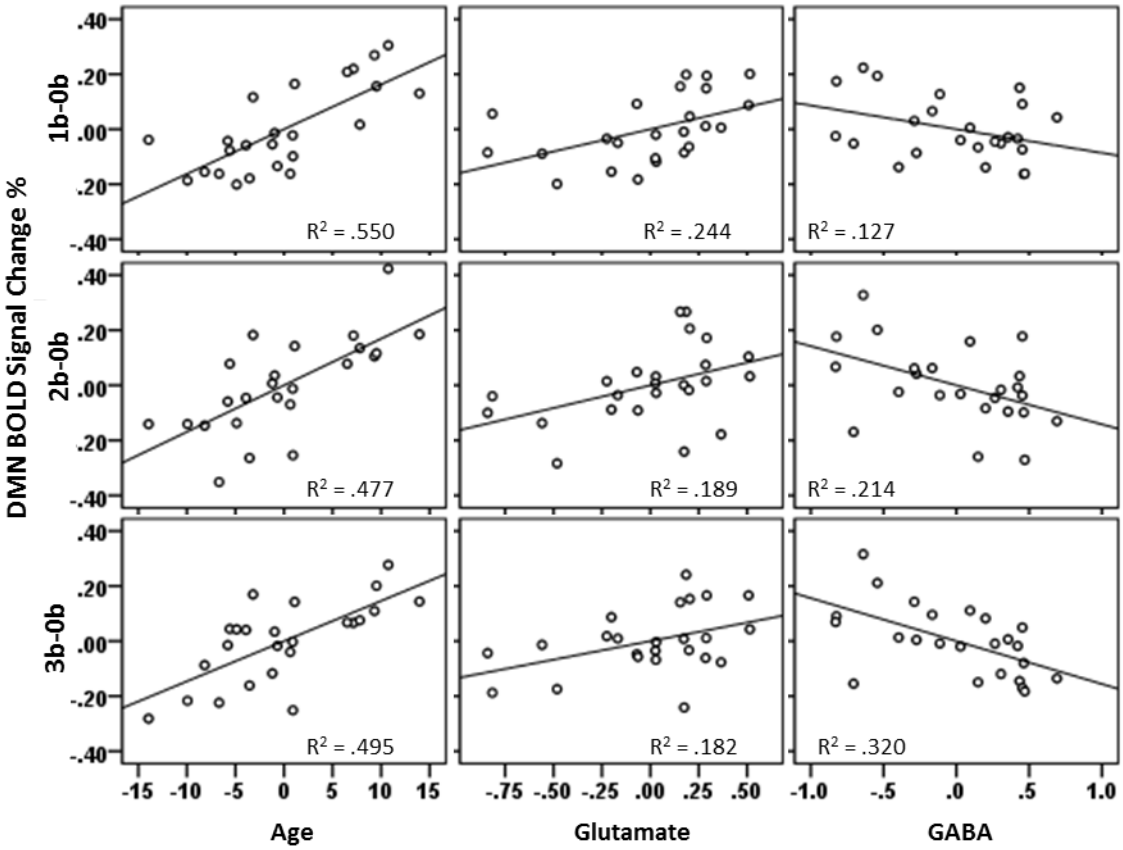

Figure S4. Associations between entire DMN BOLD signal change and age, glutamate and GABA.

Table S1 Models to regress entire DMN BOLD signal change on age, gray matter (GM), GABA and glutamate (Glu)

Model	R^2	Adj. R^2	ΔR^2	ΔF	p
			1b		
1	.418	.362	.418	7.528	.003
2	.579	.490	.161	3.631	.046
			2b		
1	.303	.237	.303	4.563	.023
2	.503	.398	.200	3.821	.040
			3b		
1	.276	.208	.276	4.012	.033
2	.537	.440	.261	5.362	.014

Model 1. Predictors: (Constant), Age, GM

Model 2. Predictors: (Constant), Age, GM, Glu, GABA

Dependent Variable: DMN BOLD signal change

Table S2 Coefficients of regression models (DMN)

Model		Beta(Std.Err)	Beta'	t	Sig.
		1b			
1	Age	.013(.003)	.675	3.675	.001
	GM	.277(.667)	.076	.416	.682
2	Age	.016(.003)	.858	4.817	<.001
	GM	-.519(.668)	-.143	-0.777	.447
	Glu	.162(.065)	.464	2.477	.023
	GABA	-.086(.052)	-.268	-1.659	.113
		2b			
1	Age	.013(.004)	.601	2.991	.007
	GM	.703(.807)	.175	.872	.393
2	Age	.017(.004)	.806	4.167	.001
	GM	-.175(.803)	-.043	-0.218	.830
	Glu	.165(.078)	.428	2.104	.049
	GABA	-.142(.062)	-.399	-2.272	.035
		3b			
1	Age	.011(.004)	.580	2.833	.010
	GM	.852(.712)	.245	1.196	.245
2	Age	.015(.003)	.804	4.312	<.001
	GM	.086(.671)	.025	0.129	.899
	Glu	.135(.066)	.403	2.056	.054
	GABA	-.156(.052)	-.506	-2.990	.008

Dependent Variable: DMN deactivation during working memory task

Model 1. Predictors: (Constant), Age, GM

Model 2. Predictors: (Constant), Age, GM, Glu, GABA

Beta(Std.Err): Unstandardized coefficient (Standard Error)

Beta' : Standardized coefficient

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